Acknowledgements: The authors acknowledge the financial support of PRIN 2008SW44CS_004. S.R. was recipient of a fellowship from INBB, Rome.

1374-Pos Board B284

Unitary Calcium Current Through Recombinant Mammalian Type 3 IP3 Receptor Channels Under Physiological Ionic Conditions

Horia Vais, J. Kevin Foskett, **Don-On Daniel Mak**.

The ubiquitous inositol 1,4,5-trisphosphate (IP_3) receptor (IP_3R) channel, localized primarily in the ER membrane, releases Ca^{2+} into the cytoplasm upon binding IP_3, generating and modulating intracellular Ca^{2+} signals that regulate numerous physiological processes. Together with the number of channels activated and the open probability (P_o) of the active channels, the size of the unitary Ca^{2+} current (i_{Ca}) passing through an open IP_3R channel determines the amount of Ca^{2+} released from the ER store, and thus the amplitude and the spatial and temporal nature of Ca^{2+} signals generated in response to extracellular stimuli. Despite its significance, i_{Ca} for IP_3R channels in physiological ionic conditions has not been directly measured. Here we report the first measurement of i_{Ca} through an IP_3R channel in its native membrane environment under physiological ionic conditions. Nuclear patch-clamp electrophysiology with rapid perfusion solution exchanges was used to study the conductance properties of recombinant homotetrameric rat type 3 IP_3R channels. Within physiological ranges of free Ca^{2+} concentrations in the ER lumen ($[Ca^{2+}]_{ER}$), free cytoplasmic $[Ca^{2+}]$ ($[Ca^{2+}]_i$) and symmetric free $[Mg^{2+}]$ ($[Mg^{2+}]_f$), the i_{Ca}-$[Ca^{2+}]_{ER}$ relation was linear with no detectable dependence on $[Mg^{2+}]_f$. i_{Ca} was 0.15 $\pm$ 0.01 pA for a filled ER store with 500 μM $[Ca^{2+}]_{ER}$. The i_{Ca}-$[Ca^{2+}]_{ER}$ relation suggests that Ca^{2+} released by an IP_3R channel raises $[Ca^{2+}]_i$ near the open channel to ~ 13-70 μM, depending on $[Ca^{2+}]_{ER}$. These measurements have implications for the activities of nearby IP_3-liganded IP_3R channels, and they confirm that Ca^{2+} released by an open IP_3R channel is sufficient to activate neighboring channels at appropriate distances away, promoting Ca^{2+}-induced Ca^{2+} release.

1375-Pos Board B285

IP3 Receptor Channels are Clustered Before IP3 Exposure with No Discernible Effect on Single-Channel Gating Properties

Horia Vais, J. Kevin Foskett, **Don-On Daniel Mak**.

A recent patch clamp study reported that recombinant rat type 3 IP_3R Ca^{2+} release channels expressed in IP_3R deficient DT40-KO cells are randomly distributed in the ER/outer nuclear membrane until exposure to low [IP_3] induces their rapid clustering [Rahman et al (2009) *Nature* **458**: 655-659]. In sub-optimal ligand conditions, clustered channels gated identically and independently, but with a lower open probability (P_o) than lone channels regardless of cluster size. In contrast, in optimal ligand conditions, P_o for clustered and lone channels were the same, but positive cooperative gating was consistently detected in patches with two active channels. Seeking to verify these surprising observations, we acquired current records from the same channels expressed in the same cell system with identical protocols and ligand conditions. The records were analyzed with the same algorithm to characterize gating behaviors of lone and clustered channels separately. For comparison, *all* nuclear patch-clamp current records previously acquired under comparable ligand conditions for recombinant rat type 3 IP_3R channels expressed in *Xenopus* oocytes, endogenous *Xenopus* type 1 IP_3R channels in oocytes and endogenous IP_3R channels in insect Sf9 cells were similarly analyzed. We found that rat type 3 IP_3R channels in DT40-KO cells are clustered without exposure to IP_3, like all IP_3R channels investigated before. For all IP_3R channels examined, we detected no significant differences between channel P_o of lone and clustered channels, in sub-optimal or optimal ligand conditions. Furthermore, in two-channel current records, the same pattern of channel gating was detected, in both sub-optimal and optimal ligand conditions, with only a small fraction (< 15%) revealing positive cooperative gating behavior. Thus, single channel behavior in all ligand conditions is independent of whether the IP_3R channel is in single or multi-channel patches.

Motions of the Cell Surface Molecules

1376-Pos Board B286

Impact of Anomalous Diffusion on Biochemical Kinetics

Marcel Hellmann, Matthias Weiss, Dieter W. Heermann.

Biochemical reactions ultimately rely on the (repetitive) encounter of two or more molecules. Diffusive transport of the reaction partners not only determines the first encounter but also the subsequent time window during which the proteins stay near to each other and repeatedly collide. Experimental observations have highlighted that diffusion in crowded media like the cell's cytoplasm or the plasma membrane is strongly anomalous, i.e. the area that is explored by a particle grows less than linearly in time. We show here that anomalous diffusion can dramatically alter the time course of biochemical reactions. In general, the behavior deviates from the classical Smoluchowski result and shows signatures of anomalous, fractal-like kinetics. While single-step reactions are typically slowed down by anomalous diffusion, multi-step reactions (e.g. phosphorylation cascades) can also be enhanced. Hence, biochemical networks in living cells could have emerged in a way to deal with or even exploit anomalous subdiffusion.

1377-Pos Board B287

A new Paradigm in Single-Particle Tracking in Live Cells: Onset of Ergodicity Breaking

Diego Krapf, Aubrey V. Weigel, Blair Simon, Michael M. Tamkun.

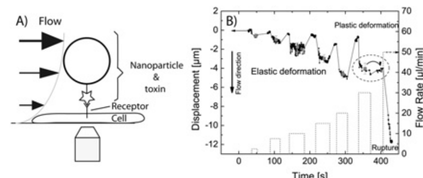
Experimental single-particle tracking (SPT) is extensively used to study the dynamics of membrane proteins and lipids in living cells. Most studies show that molecular motion in the plasma membrane, as well as in the cytoplasm and the nucleus, undergoes anomalous subdiffusion. SPT data is usually analyzed in terms of the temporal mean square displacement because averaging along individual trajectories is more readily available than ensemble averages. However, in some intriguing physical phenomena ergodicity is broken and thus temporal averages do not converge to the ensemble measurements. Here we measured more than 1,000 Kv2.1 potassium channel trajectories in live human embryonic kidney (HEK) cells, analyzed their ergodic and non-ergodic properties and uncovered the physical mechanism underlying their anomalous diffusion pattern. We have labeled Kv2.1 channels with quantum dots (QDs) in HEK cells, imaged the cell basal membrane with total internal fluorescence microscopy and analyzed the individual channel trajectories. We found that the distributions of the two types of averages are clearly different. The temporal average yielded a much broader MSD than the ensemble-average. Nevertheless, the diffusion pattern of these channels follows anomalous subdiffusion in the lag time, i.e. the MSD is sublinear, both for the temporal and the ensemble averages. Our data reveal that two processes simultaneously coexist and only one of them is ergodic. The ergodicity breaking is found to be maintained by a set of anchoring points associated to the actin cytoskeleton. Control experiments indicate these effects are not induced by the quantum dots. These results are accurately modeled by a continuous time random walk on a fractal structure. When the actin cytoskeleton is disrupted, ergodicity is recovered. These experimental observations have direct biological implications in the dynamics of membrane proteins.

1378-Pos Board B288

Single Molecule Tracking Under an External Force Field Created by Amplifying Hydrodynamic Drag with a Nano-Parachute

Silvan C. Türkcan, Jean-Marc Allain, Michel R. Popoff, Antigoni Alexandrou.

The membrane architecture plays a crucial role in many cellular processes. In this work we track the motion of two membrane receptors in an induced external force field that distorts the thermal equilibrium and gives access to the receptor interaction with the environment. We introduce a scheme that is easier to use and cheaper than optical tweezers and allows multiplexed measurements. Lanthanide doped nanoparticles (Y0.6Eu0.4VO4) are coupled to two different bacterial pore-forming toxins. Single-molecule tracking (SMT) of receptor-bound labeled toxins in the membrane of MDCK cells reveals that the receptors undergo confined diffusion in stable domains. We take advantage of the hydrodynamic interaction of labeled receptors with a controlled fluid flow within a microfluidic channel to apply a force on the receptors (Figure 1A). The nanoparticle label acts as a parachute and increases the hydrodynamic interaction with the fluid, so that drag induced by convection becomes important. In B), SMT shows mainly elastic displacements of the receptor over distances up to 10 times the confining domain diameter. Once the flow stops, the receptors return to their initial position indicating attachment to the cytoskeleton.



1379-Pos Board B289

Diffusion Measurements on Non Flat Surfaces Require Analysis of the Shortest Within Surface Distance

Ingela Parmryd, Jeremy Adler.

Diffusion in cell plasma membranes is appreciably slower than in artificial membranes, which we suspect is an artefact resulting from 2D tracking over a non-flat surface and the consequent underestimation of the net movement (1). We have compared different methods for analyzing tracks. Simple diffusion was simulated over flat and non-flat surfaces created on a 3D orthogonal grid using an array of connected voxels. On non-flat folded surfaces analyses